Microspheres for Local Drug Delivery

Zandstra, Jurjen

Chapter 1

Introduction and aim of this thesis

Kidney failure

Kidney failure is a condition in which the kidneys fail to adequately filter waste products from the blood. The two main forms are acute kidney injury (AKI), which is often reversible with adequate treatment, and chronic kidney disease (CKD), which is often not reversible. Both forms of kidney failure are characterized by a decrease in glomerular filtration rate, the rate at which blood is filtered in the glomeruli of the kidney[1,2]. This is detected by a decrease or absence of urine production, or by determining waste products (creatinine or urea) in the blood. In AKI a rapid and progressive loss of renal function is seen, generally characterized by oliguria (decreased urine production), fluid and electrolyte imbalance[3]. To arrest the progress of kidney failure the underlying causes must be identified and treated, and dialysis may be necessary to bridge the time gap required for treating these causes.

Unfortunately CKD still develops in a significant percentage of AKI patients[4]. As kidney disease progresses, the accumulation of waste products in the blood may lead to complications such as anemia and weak bones[5,6]. Furthermore, a major complication of CKD is the risk of developing cardiovascular-related diseases[7]. These problems may develop slowly over a long period of time. CKD mostly leads to kidney failure, requiring dialysis or a kidney transplant to maintain life.

In the U.S.A. alone, there are currently 26 million people with CKD (National Kidney Foundation New York 2012) and 571.000 patients with end stage renal disease who are waiting for a kidney transplant (U.S Renal Data System 2011). The incidence of diabetes, one of the main risk

factors for developing kidney disease, is also increasing, implicating that the number of kidney patients will only increase in the coming years.

In this thesis we propose, as there is no cure to halt CKD, to focus on AKI to prevent these patients to progress to CKD. We hypothesize that there is a therapeutic time window in the acute phase and we believe that when the disease is modulated and treated well, these patients will be at minimum risk to develop CKD.

Causes

AKI occurs when the blood supply to the kidneys is suddenly interrupted or when the kidneys are overloaded with toxins. Causes of AKI include accidents, injuries, and complications from surgery in which the kidneys are deprived of normal blood flow for extended periods of time (ischemic injury). Cardiac bypass surgery[8,9] is an example of one such procedure, but such a period of ischemia can also happen during kidney transplantation. People suffering from AKI require supportive treatment until their kidneys recover function. Unlike the kidneys of CKD patients, the kidneys can recover from AKI, allowing the patient to resume a normal life, but an increased risk of developing future kidney failure may still remain[10]. Thus, even if it seems as if the AKI patient has been cured, a progressive renal deterioration may continue undetected.

We hypothesize that modulating the kidney's hostile microenvironment (caused by the AKI) may prevent the progression towards kidney failure later on in life. If so, there is a window of opportunity for these AKI patients, namely after the acute phase is over, but before the kidneys

have become fibrotic, to modulate the kidney's microenvironment in such a way that these patients do not develop further kidney failure.

Cellular processes in kidney injury

Immune cells are important contributors to ischemic kidney injury and repair. Neutrophils attach to endothelium, which is activated by oxygen deprivation, and produce proteases, myeloperoxidases, reactive oxygen species, and cytokines. This leads to increased vascular permeability and reduces tubular epithelial and endothelial cell integrity, aggravating kidney injury[11]. Different types of macrophages have been described in the past few years, of which the classically activated (pro-inflammatory) and alternatively activated (pro-repair) macrophages are best known. Classically activated macrophages produce large amounts of inflammatory cytokines, while alternatively activated macrophages are mainly involved in damage repair by releasing growth factors and anti-inflammatory cytokines such as IL-10. Classically activated macrophages are known to be detrimental in the onset of AKI, whereas alternatively activated macrophages may play an important role during tissue repair[12,13].

AKI can also lead to incomplete tubular repair, tubulo-interstitial inflammation, proliferation of fibroblasts, and excessive deposition of extracellular matrix, which eventually may lead to fibrosis. The excessive collagen formation is caused by myofibroblasts which develop predominantly from perivascular fibroblasts (pericytes). The development of fibrosis after acute tubular injury has important clinical consequences such as destruction of the nephrons and impairment of overall kidney

function[14].

The various pathogenic mechanisms that lead to CKD converge to a common pathway that results in progressive interstitial fibrosis, peritubular capillary loss with hypoxia, and destruction of functioning nephrons because of tubular atrophy. Excessive extracellular matrix production occurs primarily by myofibroblasts. Impaired activity of the endogenous renal matrix-degrading proteases may enhance interstitial matrix accumulation, but the specific pathways that are involved remain unclear. Tubular epithelial cells, inflammatory cells, and myofibroblasts themselves synthesize the molecules that activate fibrogenic cascades, the most important molecule being transforming growth factor β (TGF-β). TGF-β may direct cells to assume a profibrotic phenotype and stimulate synthesis of other fibrogenic molecules. Reduced levels of antifibrotic factors that are normally produced in the kidney such as hepatocyte growth factor and bone morphogenic protein-7 may accelerate fibrosis finally leading to kidney failure[15].

Treatments

There is no specific pharmacologic therapy proven to treat AKI. Current treatment options are based on removal of the cause of AKI. Several promising experimental drugs against AKI itself have been proposed, such as growth factors, vasoactive peptides, adhesion molecules and endothelin inhibitors. However, these experimental drugs, when delivered systemically may have severe systemic side effects[16]. Therefore we believe that local drug delivery is the preferred method in these cases, as we expect

that it increases therapeutic effects on the affected tissue while minimizing systemic side effects. In addition, drugs that have a low half-life or high degradation rates in blood are not suitable for normal systemic treatment can successfully be administered locally.

Before the hypothesis can be tested, a tool is needed for the local delivery of the drug. Such a tool could typically be a biomaterial carrier (device) capable of releasing the drug of interest. We called this tool: Device for Smart Intervention in Renal rEpair (DESIRE). The most important characteristics of such a device are that it is (1) biocompatible, (2) capable of releasing a drug controllably in time, and (3) injectable for application purposes.

Local drug release

Local release of drugs allows for delivery of the largest fraction of drug molecules at or near the site where it is needed, which reduces off-target drug toxicity[17,18]. Local delivery is therefore an attractive alternative to systemic delivery, which can produce adequate doses of the agent in target tissue, as well as reduce toxicity in healthy tissue. As a result, implantable or injectable polymeric delivery systems are widely studied for the local treatment for e.g. brain tumors[19,20], vascular diseases[21,22], ocular diseases[23-26], and wound healing[27-29]. This delivery system preferably provides a constant local release of the drug at the disease site, without negatively impacting the healthy surrounding area. The ability to control dose and spatial penetration of the therapeutic agent increases its effectiveness locally, while minimizing toxicity to other tissues.

There are many features that contribute to an effective local controlled release. Dispersion of drugs is governed by physiological transport principles, which are particular to the anatomical site and which can influence both the rate of release of the agent from the release device and its fate in the local tissue. This dispersion is also dependent on the characteristics of the drug that is used, such as molecular size, hydrophobicity or hydrophilicity. Furthermore, the foreign body reaction (FBR) against the release device can have a critical effect upon its release. The local anatomy and the FBR are therefore potential barriers to local delivery. Understanding these barriers is important in the design of the controlled release device[30].

Foreign body reaction

The foreign body reaction (FBR), which is driven by macrophages and foreign body giant cells, is the end-stage response of the inflammatory and wound healing responses following implantation of a biomaterial. An understanding of the FBR is important since it may impact the biocompatibility, and thus the safety, of a biomaterial and may significantly impact short- and long-term tissue responses to tissue-engineered constructs. These constructs can contain proteins, cells, drugs and other biological components for use in tissue engineering and regenerative medicine. Host reactions following implantation of biomaterials include injury, blood-material interactions, provisional matrix formation, acute inflammation, chronic inflammation, granulation tissue development, fibrosis, and fibrous capsule development[31-33].

Following the initial blood/material interactions and provisional

matrix formation, acute inflammation followed by chronic inflammation occurs. The extent of these responses is, apart from material properties, also determined by the extent of injury during the implantation procedure and the tissue or organ into which the device is implanted. Neutrophils (polymorphonuclear leukocytes, PMNs) characterize the acute inflammatory response. Chronic inflammation is characterized by the presence of mononuclear cells, i.e. macrophages and lymphocytes, at the implant site. This inflammatory response to biomaterials is usually of short duration (2-4 weeks) and is confined to the implant site. With biocompatible materials, earlier resolution of the acute and chronic inflammatory responses occurs, with the chronic inflammatory response composed of mononuclear cells usually lasting no longer than two weeks[34].

Macrophages secrete growth factors and angiogenic factors that are important in the regulation of fibro-proliferation and angiogenesis, respectively[35,36]. Therefore macrophages play an important role in encapsulating a foreign body material. Alternatively activated macrophages can enhance fibrogenesis both directly by overexpressing extracellular matrix proteins[37] and indirectly by activating fibroblasts[38], as opposed to classically activated macrophages which inhibit fibrogenesis[39]. Following implantation of a biomaterial a fibrous capsule develops, which can interfere with biomaterial function, such as drug release from a biomaterial[40]. Thus the response of the cells implicated in the development of the fibrous capsule is an important consideration in the design of a biomaterial.

Aim of this thesis

The aim of this project is to develop a Device for Smart Intervention in Renal rEpair (DESIRE). We studied the requirements for such a device, namely its biocompatibility, its capability of controlled drug release, and its injectability under the renal capsule, thereby providing a local drug depot. Finally we examined whether drug release from our device would lead to increased therapeutic effects.

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